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Antibacterial effects of *Arctium lappa* and *Artemesia absinthium* extracts in laboratory conditions

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ABSTRACT

Introduction: *Arctium lappa* (Great burduck) and *Artemesia absinthium* are medicinal plants that some of their antibacterial and antivirus properties have been suggested in nutritional industries. The objective of this research was to study the effects of *A. lappa* and *A. absinthium* on some microorganisms including *Pseudomonads aeraginosa*, *Haemophilus influenza*, *Bacillus subtilis*, *Bacillus cereus*, *Klebsiella pneumonia* and *Staphylococcus aureus*.
Methods: Extracts were prepared by maceration method and tested on Mueller Hinton agar medium based on disc diffusion method. The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) were determined by micro-dilution method. Antibiotic disks used for controlling and standardizing the examination.
Results: The extracts of *A. lappa* and *A. absinthium* had significant effect on *S. aureus*. The MIC and MBC concentrations of the extract of *A. lappa* on *B. subtilis* were respectively 600 and 750 mg/ml. Also, these values were 230 and 540 mg/ml for *H. influenza*. Extract of *A. absinthium* showed more inhibitory effect on *B. subtilis*. All extracts showed inhibitory effect on *B. cereus*. The extracts of *A. lappa* and *A. absinthium* had inhibitory effects on *H. influenza and P. aeraginosa*. Among antibiotics, only *Ofloxacin* and *Ciprofloxacin* had effects on *H. influenza*. Extract of *A. lappa* showed flimsy effect on *K. pneumonia,* while extract of *A. absinthium* had no effect on this bacterium.
Conclusion: Due to the effects of *A. lappa* and *A. absinthium* on some bacteria, they might be good substitutes for synthetic substances.

Implication for health policy/practice/research/medical education:
Extracts of *Arctium lappa* and *Artemesia absinthium* have good antibacterial properties against some gram positive and negative bacteria. Nevertheless, more investigations need for identification of the active chemical compositions of these extracts responsible for their antibacterial properties as well as their certain mechanisms of accomplishment.
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Introduction

Herbal remedies utilization in order to treat diseases goes back to thousands of years in Iran and there are several scientific documents in this era (1). Medicinal plants have considerable antimicrobial activities that can be used for preventing or inhibiting growth of infectious microorganisms and degeneration factors. High interest in replacing chemical materials with natural ones caused performing thousands of studies on natural resources researches on different plants extracts resulted in discovery of suitable natural substances of treatment of various diseases (2). The therapeutic properties of extracts and essential oils against microbial and non-microbial diseases have been known from many years ago and many positive effects have been reported from different plant species against microorganisms (3,4). In recent decades, antimicrobial properties of herbal products have attracted many researchers because of a rapid increase in antibiotic resistance to microorganisms (5). Many members of the genus *Artemisia* are important medicinal plants. Previously, the

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antibacterial effects of some *Artemisia* species have been reported (6,7). *Artemisia* is one of the various genera of Asteraceae family with many important medicinal properties (8). Essential oils of *Artemisia* spp. have been widely used for different medicinal purposes for several years (9,10). *Artemisia* species have been used in folk medicine as antipyretic, antiseptic, anthelmintic, tonic and diuretic remedy (11). *A. lappa* was brought from Japan and acclimated in Brazil. It is widely used in popular medicine all over the world for its well-known therapeutic applications. It has anti-bacterial, antifungal (12), diuretic (13), anti-oxidant (14) anxiolytic (15), anti-platelet aggregating (16) and HIV-inhibitory properties (17). Bacterial resistant to antibiotics is increasing, requiring introduction of new and safe antibacterial substances. The present investigation was undertaken to evaluate the antibacterial activity of hydro-alcoholic extracts of *A. lappa* and *Artemesia absinthium* on *P. aeruginosa, H. influenza, B. subtilis, B. cereus, Klebsiella pneumonia* and *Staphylococcus aureus* in laboratory conditions.

Materials and Methods

In this research, *A. lappa* and *A. absinthium* were collected from Dare-Moradbeig region and Buali-Sina garden of Agriculture and Natural Research Center of Hamedan in June 2012, respectively. Herbarium samples were prepared and authenticated by Dr. Kalvandi (Botanist of Agriculture and Natural Research Center). Aerial parts of *A. absinthium* (including flower, leaf and stem) and *A. lappa* (including leaf and stem) were dried in shade and powdered (18). Hydro-alcoholic (70%) extracts were prepared by Maceration method. Microorganisms (including *P. aeruginosa* PTCC1599, *H. influenza* PTCC1623, *B. subtilis* PTCC1156, *B. cereus* PTCC1247, *Klebsiella pneumonia* PTCC1290 and *Staphylococcus aureus* PTCC1337) were obtained from Fungi and Industrial Bacterial Collection of Iran (Scientific Industrial Researches Center of Iran). Standard medium was used for microorganism culture. Antibiotics (such as *Penicillin*, *Gentamicin*, *Ciprofloxacin*, *Ofloxacin* and *Erythromycin*) were obtained from Padtan Teb Company of Tehran. The microorganisms were transferred to tubes containing sterile distilled water and their turbidities were analyzed with McFarland Turbidity Standards through optic method. Then, they were inoculated in optimum mediums (19,20). Antibacterial activity was assessed by diffusion disk method. Extracts were used in different concentrations (0, 45, 90, 166, 230, 285, 333, 500, 600, 666, 714 and 750 mg/ml). Blank disks were soaked into the extract solutions and incubated in 37°C at incubator for 30 minutes. Then, the disks containing extracts were placed on culture medium in equal distances and were incubated in 37°C at incubator for 24 hours. Mean of inhibitory halos diameter of bacterial growth was measured and recorded by Murray et al (21). Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) were determined by micro dilution method (22). In order to compare the means of inhibitory halos diameter of bacterial growth, values were compared with the antibiotics ones and each test was carried out with 4 replications (21). Statistical analysis was performed using analysis of variance by SPSS version 18.0 software. Data considered significant when $P \leq 0.05$.

Results

Results showed that extract of *A. lappa* had inhibitory effect on *H. influenza* and *P. aeruginosa* (16.4 mm and 11.4 mm, respectively) (Figure 1A). Also, extract of *A. absinthium* showed inhibitory effect on *H. influenza* and *P. aeruginosa* (18.4 mm and 11.9 mm, respectively) (Table 2; Figure 2A). The MIC and MBC of *A. lappa* on *H. influenza* were obtained 230 mg/ml and 540 mg/ml, respectively. These values for extract of *A. absinthium* were 45 mg/ml and 90 mg/ml, respectively. Among antibiotics just *Ofloxacin* had effect on *H. influenza* (16.4 mm). MIC and MBC of *A. absinthium* against *P. aeruginosa* were 285 and 430 mg/ml, respectively. These values for *A. lappa* were 500 and 750 mg/ml, respectively. Ofloxacin and Gentamicin showed higher inhibitory halos diameter of bacterial growth on *P. aeruginosa*. Extract of both medicinal plants showed inhibitory effects against *B. cereus* in low concentration (166 mg/ml) and increased inhibitory effect with increasing the concentration. All antibiotics were effective on *B. cereus*, so that the highest effect was related to gentamicin (32.4 mm) and the lowest effect was related to *Penicillin* (10.4 mm). The MIC of *A. lappa* against *B. cereus* was obtained in 166 mg/ml and inhibitory effect increased with higher concentrations. Extract of *A. lappa* did not show MBC in studied concentrations. The MIC and MBC of *A. absinthium* were obtained to be 166 and 440 mg/ml, respectively. *A. lappa* showed inhibitory effect on *B. subtilis* less

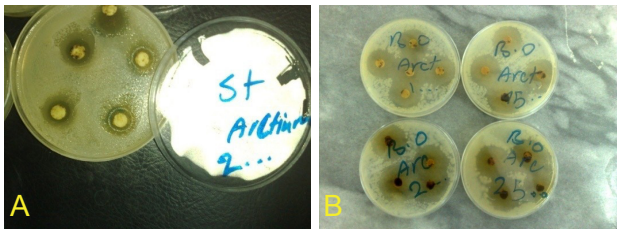

Figure 1. Effect of *Arctium lappa* on (A) *Haemophilus influenza* and (B) *Staphylococcus aureus*.

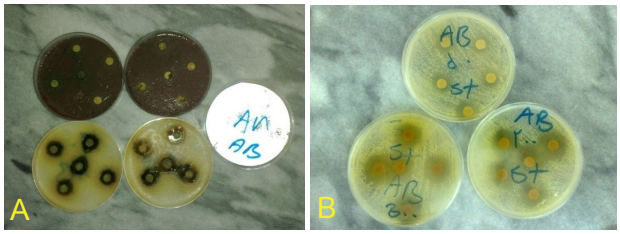

Figure 2. Effect of *Artemisia absinthium* on (A) *Haemophilus influenza* and (B) *Staphylococcus aureus*.

Table 1. Mean of inhibitory halos diameter of Bacteria growth based on used antibiotic in this study

Bacteria	Antibiotic				
	P	GM	CP	OF	ER
Haemophilus influenzae	-	-	26.4	16.4	-
Pseudomonads aeraginosa	-	26.4	36.4	20.4	-
Staphylococcus aureus	-	-	22.4	14.4	-
Klebsiella pneumoniae	-	-	16.4	18.4	-
Bacillus subtilis	-	20.4	32.4	18.4	-
Bacillus cereus	10.4	32.4	30.4	28.4	30.4

P: Penicillin (10 µg), GM: Gentamicin (10 µg), CP: Ciprofloxacin (5 µg), OF: Ofloxacin (5 µg), ER: Erythromycin (5 µg).

than *A. absinthium* (based on inhibitory halos diameter of bacteria growth). The MIC and MBC of *A. absinthium* on *B. subtilis* were 600 and 750 mg/ml, respectively, obtained by micro dilution method. These values for *A. absinthium* were 166 and 430 mg/ml, respectively. *Gentamicin* (20.4 mm), *Ofloxacin* (18.4 mm) and *Ciprofloxacin* (32.4 mm) had effect on *B. subtilis*, but *Penicillin* and *Erythromycin* had no effect. *A. lappa* and *A. absinthium* showed significant effect on *Staphylococcus aureus* ($P<0.01$) (Figures 1 and 2B). MIC of *A. absinthium* on *Staphylococcus aureus* was 230 mg/ml. *Ofloxacin* developed 14.4 mm inhibitory halos diameter of bacterial growth, while extract of *A. absinthium* in concentration of 666 mg/ml (14.9 ± 1.91) and 750 mg/ml (17.4 ± 1.15) showed higher inhibitory halos diameter (Tables 1 and 2). Different concentrations of *A. absinthium* extract had no effect on *Klebsiella pneumonia*, while MIC and MBC of *A. lappa* against *Klebsiella pneumonia* were obtained 666 and 750 mg/ml, respectively. *Ofloxacin* and *Ciprofloxacin* were able to inhibit this *bacterium* (18.4 mm and 16.4 mm, respectively) (Table 2).

Discussion

From past decades, anything that was used as drug, obtained from natural resources and mainly from plants. Using of chemical drugs developed acute problems such as auto safety due to continues taking and without caring of certain taking drug and beside effects that some of them were more dangerous than diseases. On the other hand, in recent years, microorganism resistance to chemical drugs and undesirable beside effects resulted to has been attended using of extracts and essential oils of medicinal plant that showed antibacterial effects (23). Bactericidal effects of studied plants extracts against *H. influenzae* were equal to *Ofloxacin*.

Based on the results, it was determined that sensitivities of gram positive bacteria like *Staphylococcus aureus* and *B. cereus* compared with gram negative bacteria such as *P. aeruginosa* and *Klebsiella pneumonia* to plants extracts were higher (Table 1). It has been demonstrated that *A. lappa* has antimicrobial activity against oral microorganisms. Three forms of the extract of this plant (20% tincture, extract concentrated by rotary-evaporation and lyophilized extract) were examined and reported that the lyophilized extract was the most effective against *B. subtilis* and *C. albicans* (24). In another research study it was shown that essential oil of *S. amanica* affected on all positive gram bacteria except *Staphylococcus epidermidis*. Also, these components just inhibited 2 negative gram bacteria like *Chryseomonas luteola* MU 65 and *Stenotrophomonas maltophilia* MU 64 (25). Similar results were obtained in this research from *A. absinthium* against this bacterium (Table 1).

Higher resistance in gram negative bacteria can be attributed to external phospholipid membrane that is infiltrated to lipophilic components. Lack of this membrane in gram positive bacteria causes easier entrance of the essential oil and extract components to bacterium. This process may cause increase in ionic permeability and permeation of vital inner cellular components which finally result in damage to enzyme system of bacterium. Other researchers have proved inhibition of growth of *Staphylococcus aureus, B. cereus, Klebsiella pneumonia sub sp. Pneumonia, Pseudomonas aeruginosa* and *B. subtilis* by medicinal plants (26,27). The higher effect on *H. influenza* was obtained by extract of *A. absinthium*. Extract of *A. lappa* had effect on this bacterium similar to *Ofloxacin*, while *A. absinthium* was better than *Ofloxacin*. Similar results about *A. lappa* have been obtained showing inhibitory effect on *P. aeruginosa, B. cereus* and *B. subtilis* (28). In comparison with studied medicinal plants, the effects of essential oil of different medicinal plants have been evaluated which showed positive effects on different microorganisms (29). Therefore, these plants might be suitable to be used instead of synthetic drugs. However, more studies are needed to establish the effective and safe use of these plant extracts.

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Authors' contributions

MR contributed to the conception of the work, conducting the study, revising the draft, approval of the final version of the manuscript, and agreed for some aspects of the work. RH contributed to the conception of the work, approval of the final version of the manuscript, and agreed for some aspects of the work.

Conflict of interests

Authors declare no conflict of interests.

Ethical considerations

Ethical issues (including plagiarism, misconduct, data fabrication, falsification, double publication or submis-

Table 2. Average diameter (mm) of inhibition halos produced by *Arctium lappa and Artemesia absinthium* Extracts on the target microorganisms

Medicinal plant (Extract)	Bacterium	Concentration (mg/ml)											
		0	45	90	166	230	285	333	500	600	666	714	750
Arctium lappa	*Haemophilus influenzae*	0±0	0±0	0±0	0±0	4.7±1.63	9.9±1.91	10.4±1.63	11.9±1.00	13.4±2.00	14.4±0.00	14.4±0.00	16.4±0.00
	Pseudomonads aeraginosa	0±0	0±0	0±0	0±0	0±0	0±0	0±0	2.1±1.00	8.4±0.00	9.4±1.15	10.9±1.00	11.4±1.15
	Staphylococcus aureus	0±0	0±0	0±0	0±0	0±0	0±0	6.3±1.00	9.9±1.00	11.4±1.15	14.9±1.91	16.9±1.00	17.4±1.15
	Klebsiella pneumoniae	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0	4.2±0.50	8.4±0.00	9.9±1.00
	Bacillus subtilis	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0	4.2±0.50	6.3±1.00	8.4±0.00	9.4±1.15
	Bacillus cereus	0±0	0±0	0±0	4.2±1.00	5.2±1.00	9.4±1.15	11.4±1.15	13.9±1.00	17.4±1.15	18.9±1.00	19.4±1.15	21.4±1.15
Artemesia absinthium	*Haemophilus influenzae*	0±0	2.1±0.00	2.1±0.00	6.3±0.60	9.4±1.15	9.4±1.15	9.4±1.15	10.9±1.00	16.4±0.00	16.9±1.00	17.4±1.15	18.4±1.63
	Pseudomonads aeraginosa	0±0	0±0	0±0	0±0	0±0	4.2±0.50	6.8±1.00	10.4±1.15	11.9±1.00	11.9±1.00	11.9±1.00	11.9±1.00
	Staphylococcus aureus	0±0	0±0	0±0	0±0	2.1±0.50	9.4±1.15	10.4±0.00	11.9±1.00	13.4±1.15	13.9±1.91	15.4±2.00	15.9±1.00
	Klebsiella pneumoniae	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0
	Bacillus subtilis	0±0	0±0	0±0	0±0	2.1±0.50	2.1±0.50	8.4±0.00	9.4±1.15	10.4±0.00	11.4±1.15	13.4±1.15	14.4±0.00
	Bacillus cereus	0±0	0±0	0±0	4.2±0.50	8.9±1.00	9.4±1.15	10.9±1.00	15.4±1.15	16.4±1.63	16.4±1.63	20.4±1.63	20.4±1.00

sion, redundancy) have been completely observed by the authors. This work has been performed in culture medium; so ethical committee approval was not needed.

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